Winter Cholecalciferol Supplementation at 51°N Has No Effect on Markers of Cardiometabolic Risk in Healthy Adolescents Aged 14-18 Years

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Winter **Cholecalciferol** Supplementation at 51°N Has No Effect on Markers of Cardiometabolic Risk in Healthy Adolescents Aged 14-18 Years

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Abbreviations: 25(OH)D, 25-hydroxyvitamin D; BMI, body mass index; BMI-for-age z-score, BMIz; CVD, cardiovascular disease; HDL, high-density lipoprotein; LDL, low-density lipoprotein; vitamin D₃, cholecalciferol.

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Conflict of Interest and Funding Disclosure: Authors have no conflicts of interest to declare.
Abstract

Background: Epidemiological studies have supported inverse associations between low serum 25-hydroxyvitamin D [25(OH)D] and cardiometabolic risk markers, but few randomized trials have investigated the effect of vitamin D supplementation on these markers in adolescents.

Objective: The objective of this study was to investigate the effect of winter-time cholecalciferol (vitamin D₃) supplementation on cardiometabolic risk markers in white, healthy 14-18 year-old adolescents in the UK (51°N) as part of the ODIN Project.

Methods: In a dose-response trial, 110 adolescents (15.9±1.4 years; 43% male; 81% normal weight) were randomly assigned to receive 0, 10 or 20 μg/day vitamin D₃ for 20 weeks (October-March). Cardiometabolic risk markers including BMI-for-age z-score (BMIz), waist circumference, systolic and diastolic blood pressure, fasting plasma triglycerides, cholesterol (total, HDL, LDL and total:HDL) and glucose were measured at baseline and endpoint as secondary outcomes, together with serum 25(OH)D. Intervention effects were evaluated in linear regression models as between-group differences at endpoint, adjusted for the baseline value of the outcome variable and additionally for age, sex, Tanner stage, BMIz and baseline serum 25(OH)D.

Results: Mean±SD baseline serum 25(OH)D was 49.1±12.3 nmol/L and differed between groups at endpoint with concentrations of 30.7±8.6, 56.6±12.4 and 63.9±10.6 nmol/L in the 0, 10 and 20 μg/day groups respectively (P≤0.001). Vitamin D₃ supplementation had no effect on any of the cardiometabolic risk markers (all P>0.05), except for lower HDL (-0.12 mmol/L, 95% CI -0.21, 0.04, P=0.003) and total cholesterol (-0.21 mmol/L, 95% CI -0.42, 0.00, P=0.05) in the 20 compared to the 10 μg/day group, which disappeared in the fully adjusted analysis (P=0.27 and P=0.30 respectively).
Conclusions: Supplementation with vitamin D₃ at 10 and 20 μg/day, which increased serum 25(OH)D concentrations during the winter-time, had no effect on markers of cardiometabolic risk in healthy 14-18 year-old adolescents. This trial was registered at clinicaltrials.gov as NCT02150122.

Keywords: vitamin D, adolescents, cardiovascular risk factors, randomized controlled trial
Introduction

Despite significant improvements in treatments and survival rates, cardiovascular disease (CVD) remains an important cause of mortality and morbidity in the UK (1) and globally (2), with the economic and social burden only expected to increase with an aging population (3). Whilst some of the risk factors for CVD, such as age, sex and ethnicity, are non-modifiable, others, particularly physiological/biochemical factors such as abdominal obesity, hypertension, abnormal blood lipids, diabetes, and lifestyle factors including physical inactivity and poor dietary patterns, are prevalent and potentially modifiable, making these key targets for CVD prevention.

A role for vitamin D in CVD was postulated over 30 years ago with the recognition of seasonality in CVD mortality, attributed to variations in ultra-violet radiation (4). Experimental studies have demonstrated mechanistic roles for vitamin D in reduced triglycerides and cholesterol secretion and increased peripheral removal (5, 6), pancreatic β-cell function (7) and in the endocrine regulation of blood pressure through the renin-angiotensin system (8). However, whilst the synthesis of epidemiological (9) and prospective data (10, 11) in adult populations appear to support this association between low circulating 25-hydroxyvitamin D [25(OH)D; biomarker of vitamin D status] and markers of cardiometabolic risk and cardiac events, intervention data remains limited and inconclusive (12, 13).

With strong evidence that CVD risk factors track individually (14, 15) and potentially as a combined risk phenotype (16) from childhood to adulthood, early prediction and prevention may be key to reducing the global burden of CVD. In both high and low-middle income countries adolescent populations have been shown to express a number of recognised...
physiological and behavioural CVD risk factors (17, 18). Furthermore, adolescents are a
population group with a recognised risk of low vitamin D status (19, 20) and with data
suggesting particularly strong tracking of CVD risk factors from adolescence to adulthood
(21), adolescents may be a key target population for early prevention.

However, no consistent associations have been identified between circulating 25(OH)D and
markers of cardiometabolic risk in adolescents (22). Limited randomized controlled trials
have found daily vitamin D supplementation at 50-100 µg to reduce arterial stiffness, but not
blood pressure, in normal weight adolescents (23), and improve insulin resistance in obese
adolescents (24), but with no effect on fasting glucose, triglycerides or total, low-density
lipoprotein (LDL) or high-density lipoprotein (HDL) cholesterol concentrations in other trials
(25-27). However many of the previous trials have been conducted in small samples (n < 60)
of largely obese adolescents at latitudes of < 45°N during times when skin exposure to
sunlight will influence vitamin D status.

In a dose-response, randomized controlled trial among white, healthy 14-18 year-old
adolescents residing in the UK (51°N), we have previously shown that cholecalciferol
(vitamin D₃) supplementation at 10 and 20 µg/day during the winter-time increased serum
25(OH)D concentrations and prevented the seasonal decline seen in the placebo group (28).
The aim of the present study was to investigate the effect of vitamin D₃ supplementation on
cardiometabolic risk markers (BMI-for-age z-score, waist circumference, blood pressure,
plasma lipids and glucose) as a secondary analysis of the randomized controlled trial.
Baseline associations between serum 25(OH)D and markers of cardiometabolic risk were also
determined.
Methods

Study design and participants

The present study is a secondary analysis of a vitamin D dose-response, placebo-controlled randomized trial that was conducted within the pan-European ODIN Project (Food based Solutions for Optimal vitamin D Nutrition and health through the life cycle). The primary aim of the study was to estimate dietary requirements for vitamin D in adolescents as previously reported (28). The study was conducted in parallel with a similarly designed trial in 4-8 year-old Danish children with the same primary aim (29) and secondary analyses as reported by Hauger et al. in the current issue of the journal (30). In brief, 110 healthy, white male and female adolescents aged 14-18 years, not currently taking vitamin D containing supplements or planning a winter sun vacation, were recruited onto the study and randomly allocated to receive a daily supplement of either 0, 10 or 20 μg vitamin D₃ for 20 weeks during the winter-time (October – March) (Figure 1).

Data collection

Participants visited the University of Surrey (Guildford, UK; 51°N) on two occasions at the beginning (October 2014) and end of the trial (March 2015). At each visit, an overnight fasted blood sample was obtained by venipuncture by a trained phlebotomist between 0700 and
Participants’ standing height, weight and waist circumference were measured by standardized procedures as previously described (28). BMI-for-age z-score (BMIZ) and BMI-for-age percentiles were calculated using the WHO AnthroPlus software (31). Blood pressure of the non-dominant arm was measured using an automatic blood pressure monitor (Omron Healthcare Ltd, Kyoto, Japan) while participants were sat in an up-right position with the arm supported. Readings were repeated three times, one minute apart and an average reading was calculated. Participants’ Tanner stage was determined using a self-administered questionnaire based on the five stages of pubertal development (32). Habitual dietary vitamin D and calcium intakes were estimated using an interviewer-administered validated food frequency questionnaire (33). Physical activity was assessed using a self-administered questionnaire including items on frequency, duration and type of activity both inside and outside of school. Compliance was assessed by regular telephone interviews and by tablet count of returned supplements at the end of the trial.

Laboratory analysis

All samples were stored at -80°C prior to analysis. Serum 25(OH)D concentrations were measured by liquid chromatography-tandem mass spectrometry as previously described (28). The intra- and interassay CVs were < 5% and < 6% respectively. Fasting plasma triglycerides, total and HDL cholesterol and glucose concentrations were measured using an enzymatic automated colorimetric method (ILAB 650, Instrumentation Laboratory, Milan, Italy) at the University of Surrey. All samples were measured in duplicate and a mean value was calculated. QCs were measured in triplicate at the beginning and end of each run. The intra- and interassay CVs were < 2.5% and < 1.5% respectively. LDL cholesterol concentrations were calculated using the following formula: LDL cholesterol = total cholesterol – HDL cholesterol – (triglycerides/5) (34).
Statistical analysis

Descriptive statistics were determined for all variables and reported as mean ± SD or median (IQR) for continuous variables and frequency (%) for categorical variables. Presented values are mean ± SD unless otherwise stated. Unadjusted and adjusted linear regression analyses were conducted to investigate the associations between baseline serum 25(OH)D concentration and markers of cardiometabolic risk. Multivariate models were constructed to include adjustment for sex, age, Tanner stage, BMIz and physical activity levels. The effect of vitamin D3 supplementation was evaluated as between-group differences in the endpoint cardiometabolic risk markers, adjusted for the baseline value of the outcome variable in linear regression analyses. Additional analyses were adjusted for sex, age, Tanner stage, BMIz and baseline serum 25(OH)D concentration due to its influence on serum 25(OH)D response to vitamin D3 supplementation (28). To further verify the results, dose-response associations between changes from baseline to endpoint in serum 25(OH)D and the cardiometabolic risk markers were made with similar adjustments as described above. Model assumptions were checked by visual inspection of residual and normal probability plots. Waist circumference and plasma triglycerides were log transformed before analysis and presented estimates back transformed. To allow for explorative findings in this secondary analysis, adjustment for multiple comparisons were not made. Statistical analysis of the data was completed using SPSS for Windows (version 22.0; IBM Corporation, Armonk, NY). A P value of < 0.05 was considered significant.

The original power calculation for the randomized controlled trial was based on the slope of the relationship between total vitamin D intake and endpoint serum 25(OH)D concentration (28). For the cardiometabolic outcomes, differences of at least 0.71 standard deviations
between any of the intervention groups could be detected with a power of 0.8 and significance
level of 0.05. This corresponds to differences of 5.9 mmHg in diastolic blood pressure and
0.41 mmol/L in LDL cholesterol.

Results

Compliance and protocol adherence

Of the 110 participants recruited onto the study, a total of 105 participants completed the
intervention and 102 were included in the analysis. Five participants dropped out of the study
(4.5%); n = 2 participants were excluded from the per protocol analysis due to
commencement of fish-oil supplementation, which contained vitamin D, halfway through the
intervention period and; n = 1 participant was excluded from the analysis due to an endpoint
serum 25(OH)D concentration > 125 nmol/L, which was deemed an outlier when the
distribution of the data was inspected (Figure 1). Median (IQR) compliance was 99%
(95,100).

Baseline characteristics of study participants

The baseline characteristics of the study participants are shown in Table 1. Adolescents were
15.9 ± 1.4 years of age, 43% were male and BMIz was 0.10 ± 1.01. Eighty-one percent were
of a normal weight, 12% were overweight and 7% were obese. Serum 25(OH)D was 49.1 ±
12.3 nmol/L; 5% of adolescents had 25(OH)D concentrations < 30 nmol/L and 45% had
concentrations > 50 nmol/L.

Baseline associations between serum 25(OH)D and cardiometabolic risk markers

Baseline serum 25(OH)D was inversely associated with BMIz (β = -0.44, P < 0.001) and
waist circumference (β = -0.04, P = 0.002) after adjusting for sex, age, Tanner stage and
physical activity. An inverse association was also seen for serum 25(OH)D and triglycerides in the unadjusted analysis ($\beta = -0.01$, $P = 0.008$), but not after adjustment ($P > 0.05$). There were no other associations between serum 25(OH)D concentrations and cardiometabolic risk markers (all $P > 0.05$) (Table 2).

Effect of vitamin D$_3$ supplementation on serum 25(OH)D and cardiometabolic risk markers

From baseline, serum 25(OH)D concentrations increased to 56.6 ± 12.4 nmol/L and 63.9 ± 10.6 nmol/L in the 10 and 20 $\mu$g/day groups respectively, and decreased to 30.7 ± 8.6 nmol/L in the placebo group (all $P \leq 0.001$) as previously reported (28).

Daily vitamin D$_3$ supplementation at 10 or 20 $\mu$g/day for 20 weeks had no effect on BMIz, waist circumference, systolic and diastolic blood pressure, plasma triglycerides, total, LDL and total:HDL cholesterol and glucose (Table 3). However, those in the 20 $\mu$g/day intervention group had lower plasma HDL cholesterol of -0.12 mmol/L (95% CI -0.21, 0.04, $P = 0.003$) and lower total cholesterol of -0.21 mmol/L (95% CI -0.42, 0.00, $P = 0.05$) than those in the 10 $\mu$g/day group (Supplemental Table 1). After adjustment for sex, age, Tanner stage, baseline serum 25(OH)D and BMIz, these group differences were no longer apparent ($P = 0.27$ and $P = 0.30$ respectively) and no other between-group differences were observed (Supplemental Table 2). Furthermore, linear regression analyses of changes in serum 25(OH)D and in the cardiometabolic risk markers showed no dose-response effects (data not shown, all $P > 0.05$).

Discussion

There has been increased interest over recent years in the potential role of vitamin D in cardiometabolic health and CVD risk. In this sample of healthy, primarily normal weight,
white 14-18 year-old adolescents, serum 25(OH)D was inversely associated with BMIz and
waist circumference after adjusting for potential confounders. However daily vitamin D3
supplementation for 20 weeks at doses of 10 and 20 μg, which are in line with and cover the
range of current recommendations (35, 36), had no effect on any of the cardiometabolic risk
markers measured.

Several studies have demonstrated negative associations between circulating 25(OH)D
centrations and measures of adiposity. Consistent with the findings of the present study,
circulating 25(OH)D concentrations were associated inversely with BMI in 17 year-old
Australian adolescents (37) and 12-18 year-old Korean adolescents (38), and with waist
circumference in Korean and US adolescents in the respective National Health and Nutrition
Examination surveys (38, 39). Due to its lipophilic nature, sequestering of vitamin D in
adipose tissue, thereby reducing bioavailability, is a plausible mechanism for these
associations (40). Modest weight loss in obese children and adults has been linked with an
increase in 25(OH)D concentrations (41, 42). It has also been reported that obese participants
with a BMI > 30 kg/m² demonstrated a lower rise in serum 25(OH)D concentrations in
response to increased oral vitamin D intake and ultra-violet B exposure than those with a BMI
< 25 kg/m², suggesting reduced bioavailability of vitamin D (40). While deposition of vitamin
D in body fat is one suggested mechanism for these negative associations, reduced sun
exposure due to limited mobility or avoidance of outdoor physical activity among those who
are overweight/obese may also contribute (43). In contrast to the current study, others have
reported circulating 25(OH)D to be inversely associated with systolic blood pressure (39, 44),
glucose (44) and total and LDL cholesterol (45, 46) and positively associated with HDL
cholesterol (39, 47). A systematic review of 35 observational studies and clinical trials
concluded that there was no consistent association between 25(OH)D and lipid and glucose
concentrations in children and adolescents < 18 years, and that systolic blood pressure was inversely associated with 25(OH)D in cross-sectional studies, but not in prospective cohort studies (22). Additionally it was found that there was insufficient evidence to draw any conclusions on the effect of vitamin D supplementation on cardiometabolic health in children and adolescents.

Findings from the limited number of vitamin D clinical and randomized controlled trials on cardiometabolic markers in adolescents have been inconsistent and inconclusive. Vitamin D at doses ranging from 10-100 μg/day for between 12 weeks and 6 months have previously demonstrated no effect on fasting glucose (24, 25), insulin (25), triglycerides or total, LDL or HDL cholesterol concentrations (25, 26). However these studies have largely been conducted in small samples (n < 60) of obese adolescents of mixed ethnic groups, at latitudes ≤ 45°N during seasons when sun exposure will influence cutaneous synthesis of vitamin D. A larger winter-time trial of 323 primarily normal weight adolescents also found no effect of vitamin D supplementation at 10, 25, 50 and 100 μg/day for 12 weeks on fasting glucose, insulin or markers of insulin resistance (27). The mean baseline 25(OH)D concentrations of adolescents in these trials ranged from 49-70 nmol/L. Based on the recommendations of the Institute of Medicine, current consensus concedes that a 25(OH)D concentration of > 50 nmol/L is indicative of vitamin D adequacy, based on skeletal outcomes (35). Although a 25(OH)D adequacy threshold for non-skeletal outcomes has yet to be defined, it has been suggested that in order to demonstrate beneficial effects of vitamin D supplementation on non-skeletal health outcomes, including markers of CVD risk, trials should include only individuals with baseline circulating 25(OH)D concentration < 50 nmol/L (48). Daily vitamin D₃ supplementation of 50 μg for 16 weeks improved arterial stiffness in 18 black African American adolescents with baseline serum 25(OH)D concentrations of 33 nmol/L, although there was no effect on
systolic or diastolic blood pressure (23). In a small sample of 14 obese adolescent females with serum 25(OH)D concentrations < 50 nmol/L treated with 1,250 µg vitamin D₂ once a week for 8 weeks, a tendency towards improvement in fasting glucose was seen, from 4.9 mmol/L to 4.7 mmol/L \((P = 0.05)\) (49). However, the clinical significance of a change of this magnitude is yet to be determined. Daily 25 µg vitamin D supplementation for one month increased HDL cholesterol by 0.03 mmol/L in 10-14 year-old Iranian adolescents (50). In the present study in the baseline outcome adjusted analyses, adolescents receiving 10 µg/day vitamin D₃ had higher HDL and total cholesterol than those receiving 20 µg/day. This was most likely a chance finding and this did not remain significant in the fully adjusted analyses. HDL particle sub-class has been suggested as a biological pathway for the cardio protective effects seen in epidemiological studies in adults. Specifically, the large HDL particle sub-class has been found to have a stronger association with circulating 25(OH)D than total HDL cholesterol (51). Vitamin D may therefore have a role in promoting the formation of large HDL particles that carry cholesterol away from atherosclerotic plaque, in an important atheroprotective process known as reverse cholesterol transport (51). However, these observations need to be confirmed in intervention trials.

Due to the increasing prevalence and clustering of cardiometabolic risk factors among adolescents (18, 52), new strategies for early prevention are required. Whether vitamin D should be considered in these preventative measures remains the subject of much debate. Moreover, uncertainty remains as to the dose and duration of vitamin D supplementation needed to achieve a target 25(OH)D threshold in order to exert potential beneficial effects on cardiometabolic health. The Scientific Advisory Committee on Nutrition and Institute of Medicine recommend vitamin D dietary reference values of 10 and 15 µg/day for the UK and US population respectively based on skeletal health outcomes only, due to the respective
committees noting that a lack of consistent experimental data from randomized controlled trials could not be causally linked to non-skeletal health outcomes including CVD and hypertension (35, 36). It may well be that vitamin D intakes only above the current dietary reference values have an effect on cardiometabolic health. That current vitamin D intakes from food sources in the UK, where there is limited voluntary fortification of foods with vitamin D, are around 2-4 µg/day (53), suggests the need for targeted public health strategies to increase dietary vitamin D intakes (for review see (54)). Future randomized trials should address these challenges and consider targeting adolescents with inadequate baseline 25(OH)D concentrations (< 50 nmol/L).

The double-blind, placebo-controlled randomized design of this study is a major strength. The study was conducted during the winter-time at 51°N to ensure minimal UVB exposure, there was a low drop out rate (< 5%) and compliance was high. However the sample size of the study was based on serum 25(OH)D and therefore this study may have lacked power to detect small changes in the cardiometabolic markers measured. This study was conducted in healthy, primarily normal weight, white adolescents and so cannot be generalized to the wider adolescent population.

In conclusion, vitamin D₃ supplementation at the currently recommended dietary intakes increased winter-time serum 25(OH)D concentrations but had no effect on markers of cardiometabolic risk in healthy 14-18 year-old white adolescents.
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TJS, LT, HH, CTD, CM, SL-N and KHH designed the research; TJS, LT, SL-N and KHH conducted the research; TJS performed the statistical analysis; TJS and KHH wrote the manuscript and had primary responsibility for the final content. All authors read and approved the final manuscript.
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Figure Legends

Figure 1 CONSORT flow diagram of participant enrollment, randomization and analysis to study intervention groups.

CONSORT, Consolidated Standards of Reporting Trials.
Tables

**Table 1** Baseline characteristics of participants by intervention group

<table>
<thead>
<tr>
<th></th>
<th>0 µg/day vitamin D₃</th>
<th>10 µg/day vitamin D₃</th>
<th>20 µg/day vitamin D₃</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>n</strong></td>
<td>39</td>
<td>35</td>
<td>36</td>
</tr>
<tr>
<td><strong>Male, n (%)</strong></td>
<td>16 (41)</td>
<td>15 (43)</td>
<td>16 (44)</td>
</tr>
<tr>
<td><strong>Age, y</strong></td>
<td>15.9 ± 1.4</td>
<td>16.0 ± 1.4</td>
<td>15.9 ± 1.5</td>
</tr>
<tr>
<td><strong>Tanner stage, n (%)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>2 (5)</td>
<td>2 (6)</td>
<td>5 (15)</td>
</tr>
<tr>
<td>IV</td>
<td>22 (56)</td>
<td>15 (45)</td>
<td>13 (40)</td>
</tr>
<tr>
<td>V</td>
<td>15 (39)</td>
<td>16 (49)</td>
<td>15 (45)</td>
</tr>
<tr>
<td><strong>Height, m</strong></td>
<td>1.70 ± 0.09</td>
<td>1.69 ± 0.08</td>
<td>1.69 ± 0.08</td>
</tr>
<tr>
<td><strong>Body weight, kg</strong></td>
<td>62.6 ± 15.1</td>
<td>61.5 ± 10.2</td>
<td>60.0 ± 13.2</td>
</tr>
<tr>
<td><strong>Weight status, n (%)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal weight</td>
<td>31 (79)</td>
<td>28 (80)</td>
<td>30 (84)</td>
</tr>
<tr>
<td>Overweight</td>
<td>5 (13)</td>
<td>5 (14)</td>
<td>3 (8)</td>
</tr>
<tr>
<td>Obese</td>
<td>3 (8)</td>
<td>2 (6)</td>
<td>3 (8)</td>
</tr>
<tr>
<td><strong>Physical activity, mins/d</strong></td>
<td>42 (29, 48)</td>
<td>31 (22, 46)</td>
<td>42 (22, 48)</td>
</tr>
<tr>
<td><strong>Dietary vitamin D, µg/d</strong></td>
<td>4.0 ± 2.4</td>
<td>4.2 ± 2.7</td>
<td>3.9 ± 2.1</td>
</tr>
<tr>
<td><strong>Dietary calcium, mg/d</strong></td>
<td>909 ± 467</td>
<td>938 ± 492</td>
<td>1048 ± 609</td>
</tr>
<tr>
<td><strong>Serum 25(OH)D, nmol/L</strong></td>
<td>46.8 ± 11.4</td>
<td>49.2 ± 12.0</td>
<td>51.7 ± 13.4</td>
</tr>
</tbody>
</table>

1 Values are mean ± SD, median (IQR) or n (%), n = 110.

2 Based on BMI-for-age percentiles: ≥ 2nd - < 85th normal weight; ≥ 85th - < 95th overweight; ≥ 95th obese. Calculated using the WHO AnthroPlus software (31).

3 Based on frequency and duration of activity.

25(OH)D, 25-hydroxyvitamin D.
Table 2 Baseline associations between serum 25(OH)D and cardiometabolic risk markers in 14-18 year-old adolescents

| Risk Marker                              | Unadjusted | Adjusted² |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |
|-----------------------------------------|------------|-----------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|        |
|                                         | β          | 95% CIs   | P value| β      | 95% CIs | P value|        |        |        |        |        |        |        |        |        |        |        |        |        |        |
| BMI-for-age z-score³                     | -0.40      | -6.10, -0.17 | 0.001  | -0.44  | -0.67, -0.22 | < 0.001  |        |        |        |        |        |        |        |        |        |        |        |        |        |        |
| Waist circumference (cm)                 | -0.04      | -0.07, -0.02 | 0.001  | -0.04  | -0.07, -0.02 | 0.002  |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |
| Systolic blood pressure (mmHg)          | -0.01      | -0.03, 0.03 | 0.94  | 0.01   | -0.02, 0.04 | 0.48  |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |
| Diastolic blood pressure (mmHg)         | -0.01      | -0.04, 0.03 | 0.69  | 0.00   | -0.04, 0.03 | 0.88  |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |
| Plasma triglycerides (mmol/L)           | -0.01      | -0.01, 0.00 | 0.008 | 0.00   | -0.01, 0.01 | 0.33  |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |
| Plasma total cholesterol (mmol/L)       | 0.01       | -0.01, 0.01 | 0.78  | 0.00   | -0.01, 0.01 | 0.58  |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |
| Plasma HDL cholesterol (mmol/L)         | 0.01       | -0.01, 0.04 | 0.27  | 0.00   | -0.03, 0.03 | 0.90  |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |
| Plasma LDL cholesterol (mmol/L)         | 0.01       | -0.01, 0.02 | 0.40  | 0.01   | -0.01, 0.02 | 0.38  |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |
| Plasma total:HDL cholesterol            | -0.18      | -0.58, 0.23 | 0.40  | 0.05   | -0.33, 0.44 | 0.78  |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |
| Plasma glucose (mmol/L)                 | -0.03      | -0.06, 0.00 | 0.065 | -0.03  | -0.06, 0.01 | 0.10  |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |

¹ β coefficients represent change in the cardiometabolic risk marker per 10 nmol/L increase in serum 25(OH)D evaluated in linear regression models, n = 110. Waist circumference and plasma triglycerides were log transformed before analysis and presented estimates back transformed.

² All cardiometabolic risk markers were adjusted for sex, age, Tanner stage and physical activity. Systolic and diastolic blood pressure and plasma lipids and glucose were additionally adjusted for BMI-for-age z-score.

³ Calculated using the WHO AnthroPlus software (31).

HDL, high-density lipoprotein; LDL, low-density lipoprotein.
Table 3 Cardiometabolic markers at baseline and endpoint after 20 weeks of vitamin D₃ supplementation in 14-18 year-old adolescents

<table>
<thead>
<tr>
<th>Intervention group</th>
<th>BMI-for-age z-scores³</th>
<th>Waist circumference (cm)⁴</th>
<th>Systolic blood pressure (mmHg)</th>
<th>Diastolic blood pressure (mmHg)</th>
<th>Plasma triglycerides (mmol/L)⁴</th>
<th>Plasma total cholesterol (mmol/L)</th>
<th>Plasma HDL cholesterol (mmol/L)</th>
<th>Plasma LDL cholesterol (mmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 µg/day vitamin D₃</td>
<td>0.16 ± 1.03</td>
<td>72.9 (69.6, 80.1)</td>
<td>121 ± 10</td>
<td>67 ± 8</td>
<td>0.9 (0.6, 1.3)</td>
<td>4.0 ± 0.6</td>
<td>1.3 ± 0.2</td>
<td>2.2 ± 0.5</td>
</tr>
<tr>
<td>10 µg/day vitamin D₃</td>
<td>0.12 ± 0.97</td>
<td>73.4 (71.4, 84.7)</td>
<td>124 ± 13</td>
<td>69 ± 10</td>
<td>0.9 (0.7, 1.1)</td>
<td>3.9 ± 0.7</td>
<td>1.3 ± 0.3</td>
<td>2.2 ± 0.6</td>
</tr>
<tr>
<td>20 µg/day vitamin D₃</td>
<td>0.03 ± 1.06</td>
<td>74.6 (69.9, 81.3)</td>
<td>122 ± 10</td>
<td>67 ± 6</td>
<td>0.8 (0.7, 1.1)</td>
<td>4.0 ± 0.8</td>
<td>1.4 ± 0.2</td>
<td>2.1 ± 0.7</td>
</tr>
<tr>
<td>P value²</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10 vs. 0</td>
<td>0.33</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20 vs. 0</td>
<td>0.49</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20 vs. 10</td>
<td>0.11</td>
<td></td>
<td></td>
<td></td>
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<td></td>
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<td></td>
</tr>
</tbody>
</table>
Plasma total:HDL cholesterol

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3.0 ± 0.6</td>
<td>3.0 ± 0.6</td>
<td>2.8 ± 0.6</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Endpoint</td>
<td>3.0 ± 0.7</td>
<td>2.8 ± 0.5</td>
<td>2.9 ± 0.7</td>
<td>0.33</td>
<td>0.40</td>
<td>0.08</td>
<td></td>
</tr>
</tbody>
</table>

Plasma glucose (mmol/L)

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th></th>
<th></th>
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<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5.0 ± 0.4</td>
<td>5.0 ± 0.4</td>
<td>5.1 ± 0.4</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Endpoint</td>
<td>5.2 ± 0.6</td>
<td>5.3 ± 0.7</td>
<td>5.2 ± 0.6</td>
<td>0.46</td>
<td>0.96</td>
<td>0.44</td>
<td></td>
</tr>
</tbody>
</table>

1 Baseline and endpoint values are unadjusted mean ± SD or median (IQR).

2 *P* values from linear regression models adjusted for baseline value of the outcome, *n* = 110-102.

3 Calculated using the WHO AnthroPlus software (31).

4 Waist circumference and plasma triglycerides were log transformed before analysis and presented estimates back transformed.

HDL, high-density lipoprotein; LDL, low-density lipoprotein.
Assessed for eligibility ($n = 132$)

Excluded:
- Not meeting inclusion criteria ($n = 17$)
- Declined to participate ($n = 5$)

Randomized ($n = 110$)

**Placebo**
Allocated and received intervention ($n = 39$)

**10 µg/day**
Allocated and received intervention ($n = 35$)

**20 µg/day**
Allocated and received intervention ($n = 36$)

Discontinued intervention:
- Unwilling to comply ($n = 2$)
- Illness ($n = 1$)

Discontinued intervention:
- Unwilling to comply ($n = 2$)

Discontinued intervention:
- Unwilling to comply ($n = 0$)

Completed ($n = 39$)
- Analyzed ($n = 38$)
- Exclusion reasons:
  - Breach of protocol ($n = 2$)

Completed ($n = 33$)
- Analyzed ($n = 32$)
- Exclusion reasons:
  - Breach of protocol ($n = 1$)

Completed ($n = 33$)
- Analyzed ($n = 32$)
- Exclusion reasons:
  - Outlier ($n = 1$)

Exclusion reasons:
- Breach of protocol ($n = 2$)
- Breach of protocol ($n = 1$)

Allocation

Enrolment

Follow-up

Analysis

Assessed for eligibility ($n = 132$)

Excluded:
- Not meeting inclusion criteria ($n = 17$)
- Declined to participate ($n = 5$)

Randomized ($n = 110$)

**Placebo**
Allocated and received intervention ($n = 39$)

**10 µg/day**
Allocated and received intervention ($n = 35$)

**20 µg/day**
Allocated and received intervention ($n = 36$)

Discontinued intervention:
- Unwilling to comply ($n = 2$)
- Illness ($n = 1$)

Discontinued intervention:
- Unwilling to comply ($n = 0$)

Completed ($n = 39$)
- Analyzed ($n = 38$)
- Exclusion reasons:
  - Breach of protocol ($n = 2$)

Completed ($n = 33$)
- Analyzed ($n = 32$)
- Exclusion reasons:
  - Breach of protocol ($n = 1$)

Completed ($n = 33$)
- Analyzed ($n = 32$)
- Exclusion reasons:
  - Outlier ($n = 1$)

Exclusion reasons:
- Breach of protocol ($n = 2$)
- Breach of protocol ($n = 1$)